PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Alagu P. THIRUVENGADAM, et al.

Docket No: A8709

Appln. No.: 10/823,647

Group Art Unit: 1651

Confirmation No.: 4915

Examiner: Taeyoon KIM

Filed: April 14, 2004

For: METHODS FOR DIAGNOSING A BIPOLAR DISORDER AND UNIPOLAR DISORDER

SUPPLEMENTAL DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Alagu P. Thiruvengadam, hereby declare and state:

THAT I am a citizen of the United States of America;

THAT I have received a Ph.D. degree in 1962 from Indian Institute of Science;

THAT I am a named inventor of the application;

THAT I am familiar with the disclosure and claims of the above-identified patent application;

THAT I am also familiar with the Office Action dated November 9, 2007, in the above-identified application wherein the Examiner, *inter alia*, rejects claims 27-19, 31, 32 and 45 under 35 U.S.C. § 103, as being unpatentable over El-Mallakh in light of Garrahan or Antia; and

The following supplements my Declaration filed August 31, 2007, and provides further clarification of the experiments performed therein.

The results in my previous Declaration demonstrated that there was no significant difference between the membrane potential of control and bipolar samples when treated with I) gramicidin in K^+-containing buffer, or II) ethacrynate in K^+-containing buffer.

I hereby state that in the experiments presented under sections "I" and "II" of my Declaration filed August 31, 2007, the membrane potential was determined by measuring cell fluorescence in the presence of a potential-sensitive dye (3,3'-dihexyloxacarbocyanine), as taught in Buss and El-Mallakh.

Thus, even if one of ordinary skill in the art would have been motivated to use K^+-free buffer, and use ethacrynate in place of gramicidin, the references cited by the Examiner still do not disclose measurement of membrane potential to distinguish bipolar patients from control subjects, as claimed in the present invention.

That is, as further evidenced below, unexpectedly significant differences could only be observed between non-bipolar subjects and bipolar patients using the claimed method which employs K^+-free buffer, and measurement of cell fluorescence in buffer which does not contain a potential-sensitive dye (3,3'-dihexyloxacarbocyanine).

As previously stated in the Declaration filed August 31, 2007, the methods used in the references cited by the Examiner had technical and scientific problems. These problems have been overcome in the present invention by developing a new reliable and accurate method that can distinguish bipolar patients and non-bipolar subjects using cells from a test human patient.

Specifically, the references cited by the Examiner possessed the following impediments listed below. Therefore, the methods disclosed by the references cited by the Examiner are

inadequate. In contrast, the claimed method showed and detected a significant difference between control and bipolar subjects using unexpectedly superior methods.

1. The method used by El-Mallakh and Buss measured the relative transmembrane potential in the presence and absence of gramicidin, and in the presence of a potential-sensitive dye. This method could not distinguish transmembrane potential in cultured lymphocytes of either euthymic patients from those of normal subjects or of bipolar patients from those of normal subjects.

2. Garrahan is relied upon for teaching the use K^+-free buffer for altering ionic gradients in the cell. However, Garrahan does not teach measurement of transmembrane potential, and much less K^+-free buffer in the diagnosis of bipolar disorder.

3. Antia is relied upon for teaching the use of ethacrynate to up-regulate sodium pump in lymphocytes. Antia does not teach measurement of transmembrane potential, or diagnosis of bipolar disorder. Antia simply employed radioligand binding assays to compare the differences in Na,K-ATPase proteins present in normal control, first-degree relatives of patients with bipolar disorder, and patients with bipolar disorder. Antia found no significant differences between the amount of Na,K-ATPase proteins in normal control and first-degree relatives of patients with bipolar disorder.

Based on the teachings of the above references cited by the Examiner, it would not have been obvious to a person of ordinary skill in the art to employ a ratio of transmembrane potentials in a method to diagnose bipolar disorder, as claimed in the present invention. In fact, based upon the teachings of the references cited by the Examiner, a person of ordinary skill in

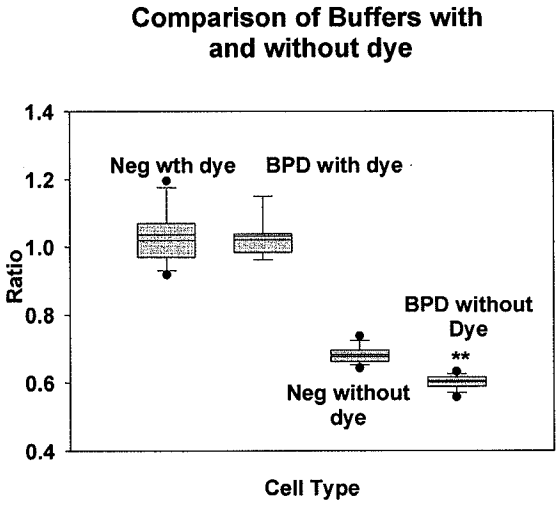
the art would have been discouraged from using a ratio of transmembrane potentials to diagnose bipolar disorder. The present invention provides a successful diagnostic method that is unexpectedly superior over the references cited by the Examiner because the claimed method overcomes the scientific and technical problems disclosed in the cited references.

Specifically, the claimed method measures the ratio of membrane potential in K^+-free buffer with a compound that alters Na,K ATPase activity (e.g., ethacrynate) to the membrane potential in buffer containing K^+ and without ethacrynate, where both membrane potentials are determined by measuring cell fluorescence in the absence of a potential-sensitive dye (3,3'-dihexyloxacarbocyanine). The use of K^+-free buffer, and absence of a potential-sensitive dye when measuring cell fluorescence in the claimed method provided a reliable and accurate technical assay. In the methods disclosed in El-Mallakh and Buss, measurement of fluorescence intensity as an indicator of membrane potential was done without removing the dye after incubation with the dye. This lack of removal masks the cellular fluorescence due to the high background of the fluorescence of the dye in the buffer. As a result, El-Mallakh and Buss were unable to differentiate the membrane potentials in the control and bipolar samples.

In contrast, a significant and unexpected technical advance was made in the present invention that was counter-intuitive to one of ordinary skill in the art at the time the invention was made, by using K+-free buffer and removing the dye from the buffer before measuring cell fluorescence. Using these unexpected technical advances, membrane potential differences between bipolar patients (both hospitalized and euthymic patients) were found in the present invention to be distinguished from that of non-bipolar subjects.

The following experiments, which were conducted by me or under my supervision, provide comparative data to show the major technical advantage offered by the claimed method, employing K^+-free buffer and removal of potential-sensitive dye (3,3'-dihexyloxacarbocyanine) before measuring cell fluorescence, provides an accurate and reliable measurement of membrane potential and enables diagnosis of bipolar disorder.

As shown below, blood cells from control (Neg) and bipolar patients (BPD) were each incubated in K^+-free buffer with ethacrynate, and in buffer with K^+ but without ethacrynate. Measurement of relative membrane potential was carried out using potential-sensitive dye present in the buffer (Neg with dye, BPD with dye), and without potential-sensitive dye in the buffer (Neg without dye, BPD without dye). The ratio was then calculated according to the claimed method. The results are shown below.



As shown above, no significant difference was observed when the potential-sensitive dye was present in the buffer during measurement of cell fluorescence. In addition, the ratio was much higher due to the high background fluorescence of the potential-sensitive dye in the buffer. In contrast, the method of the claimed invention demonstrated a significant difference ($p < 0.001$) between the groups, Neg without dye and BPD without dye, because the cells were resuspended in dye-free buffer before cell fluorescence was measured.

Thus, even if one of ordinary skill in the art would have been motivated to use K^+-free buffer, and use ethacrynate in place of gramicidin, the references cited by the Examiner do not disclose measurement of membrane potential so as to distinguish bipolar patients from control subjects as claimed in the present invention nor measurement of membrane potential in the absence of potential-sensitive dye. The results shown above demonstrates that the membrane potential ratio of non-bipolar subjects and bipolar patients were not significantly different in the presence of the potential-sensitive dye (see the left two box plot results in the figure). Significant differences could only be observed using the method claimed in the presence of the potential-sensitive dye (see the right two box plot results in the figure).

The major scientific and technical advantages that make the claimed method unexpectedly superior over other methods used to measure membrane potential, e.g., the methods disclosed by El-Mallakh and Buss, are: 1) the use of K^+-free buffer and 2) the measurement of cellular fluorescence in the absence of the potential-sensitive dye so as to eliminate background fluorescence in the buffer. These advances have not been practiced or suggested by El-Mallakh, Buss, or other investigators in the art.

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I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/7/2007

Alagu P. Thiruvengadam